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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.53(c).

Docket Number	50317/002001	Type a plus sign (+) inside this box --->	+

INVENTOR(S)/APPLICANT(S)

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TITLE OF THE INVENTION (280 characters max)

DEVICE AND METHOD FOR CONTACTING PICOLITER VOLUMES OF FLUIDS

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ENCLOSED APPLICATION PARTS (check all that apply)

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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ A check or money order is enclosed to cover the filing fees. ☒ The Commissioner is hereby authorized to charge filing fees not covered and apply any credits to Deposit Account Number: 03-2095	FILING FEE AMOUNT	$80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes, the name of the U.S. Government agency and the Government contract number are:

☒ Applicant claims small entity status under 37 C.F.R. § 1.27.

Respectfully submitted,

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PROVISIONAL APPLICATION

UNDER 37 C.F.R. § 1.53(c)

APPLICANT : Daniel Irimia, et al.

TITLE : DEVICE AND METHOD FOR CONTACTING PICOLITER VOLUMES OF FLUIDS

DEVICE AND METHOD FOR CONTACTING PICOLITER VOLUMES OF FLUIDS

Statement Regarding Federally Sponsored Research

This invention was made with Government support through the NIH. The Government has certain rights in this invention.

Background of the Invention

The invention relates to the fields of microfluidics and cellular assays.

The cell is the basic structural and functional unit of living organisms. Although all cells in one organism share the same genetic information in the form of DNA, transcription and translation differ in different types of cells. Cells are usually grouped in tissues and organs based on similar morphology and function. It is, however, increasingly recognized that many tissues are heterogenous structures, and this heterogeneity may be increased in pathological situations. As a consequence, the analysis of a sample at the single cell level may be important for diagnostic purposes. For example, only a few cells in a tumor are responsible for metastasis.

In order to obtain comprehensive pictures of cell function, simultaneous examination of the expression of thousands of genes may be necessary. This examination is made possible by the development of microarray techniques for DNA and RNA. In general, simple knowledge of the gene sequence or the quantity of gene expression may be insufficient to predict biological functions or provide appropriate diagnostic information. Thus, several techniques to enable efficient and highly parallel identification, measurement, and analysis of proteins have been developed (e.g. protein-chip array).

Most array methods require concentrations of material above a certain threshold (e.g., 2 μg at 0.02 μg/μl concentration for mRNA analysis), and few methods can work with samples as small as 1000 cells. Flow cytometry can address issues of heterogeneity at the single cell level, but only for a small number of targets, which is limited by the number of fluorescent dyes available. In the case of genes, another limitation in the use of fluorescent probes comes from the requirement for *a priori* knowledge of the sequence of interest. Consequently, it has not yet been possible to address the issue of heterogeneity using current techniques for global analysis at the single cell level.

Microfabrication techniques have shown the potential to manipulate small biological samples precisely and to improve the sensitivity of molecular detection. Previous attempts to extract genetic or protcomic information from living cells using on-chip systems has been limited to open flow systems, where the dilution of the target molecules is significant, making its identification problematic.

Thus, there is a need for new devices and methods for analyzing the contents of individual cells that have increased sensitivity.

Summary of the Invention

The invention features a device for contacting fluids and methods of use thereof. The device is based on the ability to control the flow of fluids, e.g., by contact angle and channel size. Fluids in the device can be divided to form segments of controlled volume, which are then brought together to initiate mixing. An exemplary use of the device is for the lysis of single cells. The device can be integrated on the same chip with other devices, for example, for cell handling or analysis of DNA, mRNA, and proteins released from the lysis of a cell.

In one aspect, the invention features a device for contacting two volumes of fluid. This device includes a first channel having a fluid inlet and a first fluid divider for dividing the fluid in the first channel into discrete segments; a second channel having a fluid inlet and a second fluid divider for dividing the fluid in the second channel into discrete segments; and a third channel connecting the first and second channels. The first

or second fluid divider may include one or more sources for an immiscible fluid, such as an enclosed chamber connected to the first or second channel which may be heated, e.g., by a resistive heater or a radiation source. In one embodiment, a channel in the device may contain a constriction. The contact angle of an aqueous solution with the surface of the first, second, or third channel may be greater than 90°. The surfaces of the channel may also be modified, e.g., by light or an electric field, to have a contact angle of less than 90°. In another embodiment, the first or second channel includes a chamber having a volume of 0.1 pL – 100 μL.

The device of the invention is used in a method of contacting two fluids including the steps of providing a device as described herein; pumping a first fluid through the inlet into the first channel and pumping a second fluid through the inlet into the second channel; employing the first fluid divider to divide the first fluid into a plurality of segments to form a first segment connected to the third channel; employing the second fluid divider to divide the second fluid into a plurality of segments to form a second segment connected to the third channel; and contacting the first and second segments via the third channel. In one embodiment, the first or second fluid divider, e.g., an enclosed chamber as described herein, includes one or more sources for fluid immiscible in the first or second fluid, and the immiscible fluid is introduced into the first or second channel to divide the fluid therein into a plurality of discrete segments. The contacting step may occur before, during, or after the fluid dividing steps. Segments of divided fluid have a volume, for example, of 0.1 pL – 100 μL. Constrictions or sieves in the first or second channels may be employed to trap a particle, e.g., a cell, as fluid flows through the device. The contact step occurs, for example, by reducing the pressure in the third channel relative to the first and second channels or by reducing the contact angle of the first and second fluids with the third channel to less than 90°. In other embodiments, the first fluid has a contact angle of greater than 90° with the surfaces of the first and third channels, or the second fluid has a contact angle of greater than 90° with the surfaces of the second and third channels.

Preferably, the first fluid includes a cell, and the second fluid includes a lysis solution capable of lysing the cell. In this embodiment, the first segment includes the cell, and the second segment includes the lysis solution, and the cell is lysed after the two segments are contacted.

By "discrete" is meant physically separated.

By "fluid" is meant a liquid or gas. A fluid may be a solution or suspension, and it may carry particulate matter, e.g., cells.

By "immiscible" is meant does not substantially dissolve on the time-scale of operation of a device of the invention. An exemplary immiscible fluid for use with aqueous solutions is air.

By "lysis solution" is meant a solution that will cause at least one type of cell to lyse.

By "microfluidic" is meant having one or more dimensions of less than 1 mm.

By "θ" is meant the contact angle between a fluid and a surface.

The device of the invention has the capability of mixing two complex fluids, e.g., for lysing one or more mammalian cells in a closed chamber. The volume of the chamber is sized such that the final concentration of the cellular components in the lysis chamber is advantageously comparable to their concentration inside the cell. The device of the invention also has the following features and advantages: (1) the use of fluid expansion and fluid-fluid (e.g., liquid-gas) interface to separate volumes of fluid on the order of picoliters into discrete segments; (2) the use of constrictions or sieves to capture and position a cell from a cell suspension inside the device; (3) the use of fluid-fluid (e.g., liquid-gas) interfaces to control the flow path of a fluid in connecting channels; (4) the use of fluid expansion to manipulate the separated liquid volumes and achieve active mixing; (5) the use of electric currents, or other power sources such as radiation or chemical energy, to control the fluid volume separation and mixing on the chip, without the need for external pneumatic or mechanical actuators; (6) the use of magnetic beads inside the device for capturing and manipulation of specific molecules released during cell lysis; (7) the use of optically transparent materials to allow observation of the device

by transmitted-light microscopy and similar techniques, simultaneously with cell manipulation and lysis; (8) the capability of integration with other microfluidic devices, both prior to introduction into (e.g., cell handling, incubation, or separation devices) and after introduction into (e.g., gene or protein microarray devices) the device of the invention; and (9) the capability of automation and massively parallel processing of individual samples (e.g., cells).

Other features and advantages will be apparent from the following description and the claims.

Brief Description of the Drawings

Figure 1 is an electron micrograph of a lysis device of the invention. Letters and numbers correspond to different sections of thc channels as described below.

Figure 2 is an electron micrograph of the lysing chambers and adjacent channels of the device shown in Figure 1. Cells are trapped and separated in chamber **7** while a volumc of the lysing solution is separated in chamber **11**. The fluids in chambers **7** and **11** are mixed by suction in channel **6**. The contents of the chambers **7** and **11** and channel **6** can be removed through channel **12**.

Detailed Description of the Invention

The invention features a device and method for contacting two or more volumes of fluid. For example, the device of the invention is capable of capturing a single cell and lysing it in an isolated chamber with a total volume (~ 50 – 100 pL) comparable to or within several orders of magnitude of the cell volume (~ 2 pL). In this embodiment, the molecules released by lysis are present in concentrations similar to intracellular concentrations. In other embodiments, other fluids containing, for example, samples, reagents, or synthetic precursors, may be contacted using the device and methods of the invention.

Device

The device typically contains two main channels connected by a third, connecting channel. Although some fluid from the main channels may enter the connecting channel, that fluid does not come into contact via the connecting channel without appropriate actuation. For example, when using aqueous solutions and hydrophobic channels, entry of fluid into the connecting channel is typically minimized by reducing the cross-sectional area of that channel relative to the main channels. In general, the surfaces of the main channels and connecting channels are designed such that the fluids being added to the channels maintain a contact angle (θ) of greater than 90°, such that fluids do not wet the channels by capillary action, thereby ensuring that additional pressure is needed to push fluid into those channels. Valves may also be used to control the entry of fluid into various channels or chambers. Channels in the device may have any cross-section capable of passing the fluids of interest, e.g., circular ot rectangular. The surfaces of the channels may be hydrophobic or hydrophilic depending on the nature of the fluid introduced into the main channels. The main channels may also include enlarged areas to form chambers of defined volume for mixing.

The device also includes fluid dividers for dividing the fluid in the main channels into segments by introducing a fluid into the main channels. This introduced fluid is preferably immiscible in the fluid in the main channels. The main channels may also contain constrictions to prevent fluid flow beyond a defined point, e.g., to minimize the usage of reagents or to trap particulate matter. Constrictions or sieves (e.g., a series of posts) can also be employed to trap particles in the device.

Fluid dividers may be any device that is capable of dividing a column of fluid into segments, for example, by introducing an immiscible fluid into the fluid columns in the main channels. Typically each main channel has two fluid dividers to divide the fluid in the main channel into three segments, thereby isolating the middle segment. Additional fluid dividers may be present to divide the fluid in the main channels into more than three segments, e.g., for parallel or serial processing of multiple volumes of fluid. Examples of fluid dividers include pumps that force fluid (e.g., air or a hydrocarbon) into the main channels. One exemplary fluid divider includes an enclosed chamber of fluid that has an

inlet into the main channel. When heated, e.g., by a radiation source such as a laser or a resistive heating element, fluid in the chamber expands and flows into the main channel. Preferably, the channels have a round cross-section when employing fluid dividers that introduce fluids, but other cross-sections, e.g., rectangular, may also be employed. Alternatively, a fluid divider may be a series of mechanical valves that physically divide a fluid column into segments when closed. In this case, the channel containing the segment of fluid to be manipulated further in the device preferably has an inlet (e.g., a small channel or a valve) for a fluid, e.g., air, to equalize the pressure as the fluid segment is transported in the device. In another embodiment, the fluid divider may be a heater capable of nucleating a gas bubble in the fluid column, or the divider may involve a chemical or electrochemical reaction capable of evolving gas. The amount of immiscible fluid introduced or generated into the channels by the dividers will depend on the volume of the channels in the device. Combinations of various fluid dividers may also be employed in a single device.

The device may allow for the pressure in the connecting channel to be reduced relative to the pressure in the main channels in order to cause fluids in the main channels to come into contact. This effect may occur, for example, by applying reduced pressure (e.g., suction) to the connecting channel or by increasing the pressure in the main channels. Alternatively, the θ of the fluid in the main channels with the connecting channel may be altered to cause fluids in the main channels to come into contact by application of light or an electric field to the connecting channel or introduction of additives to the fluid. The connecting channel may also be connected to an outlet channel for applying suction, removing the products of the fluids contacted in the device, and providing active mixing.

An exemplary device of the invention is shown in FIG. 1. The device includes two main channels having three inlets for fluids, **A**, **B**, and **C**. Both of the main channels include a constriction (labeled **2** in the A/B channel and **8** in the C channel). Fluid introduced via the A or B inlet may be pumped into the channel until reaching constriction **2**. The constriction in the channel may prevent the passage of particulate

matter. A third channel **6** connects the two main channels and is, in turn, connected to a channel **12** through which suction may be applied and the contents of channel **6** removed. Each of the main channels contains a chamber (labeled **7** in the **A/B** channel and **11** in the **C** channel) that houses the volume of fluid to be contacted via channel **6**. The fluid dividers are chambers **4, 5, 9,** and **10**, two of which flank each chamber **7** and **11**. Each of chambers **4, 5, 9,** and **10** contains a heater. The dimensions of the channels in the device are typically microfluidic.

In another embodiment, each main channel includes only one fluid divider, which divides the fluid column into two segments. The device may be designed such that each main channel has a constriction through which the introduced fluid cannot flow. The location of the fluid divider relative to the constriction then determines the volume of the fluid segment separated from the remainder of the fluid column.

In an alternative embodiment, the fluids to be mixed in the device have a θ of less than 90° with the surfaces of the main channels, such that the fluids wet the main channels by capillary action. The surfaces of channels connecting the main channels have a θ greater than 90°, thereby preventing the contact of the fluids in the main channel until the relative pressure of the connecting channel is reduced. In this embodiment, enclosed fluid chambers, e.g., as depicted in FIG. 1, may be used as the fluid divider. The θ of the surfaces of the enclosed chambers may also be greater than 90° in order to prevent entry of fluids from the main channels. Alternatively, the θ surfaces of the enclosed chamber may be less than 90° if the fluid in the enclosed chambers cannot be substantially displaced by the fluid in the main channels.

In another embodiment, the contacting of fluids in the connecting channels can be controlled by the length of the connecting channel. For example, fluids are introduced into the main channels and begin to flow into the connecting channel. The fluid dividers can then be operated to divide the fluid columns in the main channels. This division may occur before the fluids contact in the connecting channel, as the fluids are contacted, or

shortly after the fluids contact. The length of the connecting channel can be adjusted to control the time of contact relative to the actuation of the fluid dividers.

A device of the invention may also include more than two main channels, from which fluids may be contacted. For example, fluids from three or more channels may be contacted simultaneously or in series. In addition, the device may be designed to allow parallel or sequential contacting of fluids from fewer than all of the main channels. Such an arrangement may be two-dimensional or three-dimensional. In addition, fluid introduced into the main channels of the device may be the output from another device, e.g., a cell separator or chromatograph. The fluid produced on the device may also be directed into another device, e.g., a capillary electrophoresis chip, for further manipulation. Such additional devices may be integrated onto the same chip as the device of the invention, or the additional devices may be physically separated.

Fabrication

A variety of techniques can be employed to fabricate a device of the invention, and the technique employed will be selected based in part on the material of choice. Exemplary materials for fabricating the devices of the invention include glass, quartz, silicon, steel, nickel, poly(methylmethacrylate) (PMMA), polycarbonate, polystyrene, polyethylene, polyolefins, epoxy resins, poly(ethylene glycol), silicones (e.g., poly(dimethylsiloxane) (PDMS)), and combinations thereof. Other materials are known in the art. In one embodiment, the device is fabricated, at least in part, from a transparent material to allow for visual inspection or optical measurements (e.g., fluorescence or absorbance).

Methods for fabricating channels in these materials are also known in the art. These methods include, photolithography (e.g., stereolithography or x-ray photolithography), molding, casting, embossing, silicon micromachining, wet or dry chemical etching (e.g., reactive ion etching or deep reactive ion etching), milling, diamond cutting, Lithographie Galvanoformung and Abformung (LIGA), and electroplating. For example, for glass, traditional fabrication techniques of

photolithography followed by wet (KOH) or dry etching (reactive ion etching with fluorine or other reactive gas) can be employed. Techniques such as laser micromachining can be adopted for plastic materials with high photon absorption efficiency. This technique is suitable for lower throughput fabrication because of the serial nature of the process. For mass-produced plastic devices, thermoplastic injection molding, and compression molding is suitable. Conventional thermoplastic injection molding used for mass-fabrication of compact discs (which preserves fidelity of features in sub-microns) may also be employed to fabricate the devices of the invention. For example, the device features are replicated on a glass master by conventional photolithography. The glass master is electroformed to yield a tough, thermal shock resistant, thermally conductive, hard mold. This mold serves as the master template for injection molding or compression molding the features into a plastic device. Depending on the plastic material used to fabricate the devices and the requirements on optical quality and throughput of the finished product, compression molding or injection molding may be chosen as the method of manufacture. Compression molding (also called hot embossing or relief imprinting) has the advantages of being compatible with high-molecular weight polymers, which are excellent for small structures, but is difficult to use in replicating high aspect ratio structures and has longer cycle times. Injection molding works well for high-aspect ratio structures but is most suitable for low molecular weight polymers.

A device may be fabricated in one or more pieces that are then assembled. Layers of a device may be bonded together by clamps, adhesives, heat, anodic bonding, or reactions between surface groups (e.g., wafer bonding). Alternatively, a device with channels in more than one plane may be fabricated as a single piece, e.g., using stereolithography, multi-layer fabrication techniques, or other three-dimensional fabrication techniques.

In one embodiment, the device is made of PMMA. The features, for example those shown in Figure 1, are transferred onto an electroformed mold using standard photolithography followed by electroplating. The mold is used to hot emboss the features

into the PMMA at a temperature near its glass transition temperature (105 °C) under pressure (5 to 20 tons) (pressure and temperature will be adjusted to account for high-fidelity replication of the deepest feature in the device). The mold is then cooled to enable removal of the PMMA device. A second piece used to seal the device, composed of similar or dissimilar material, may be bonded onto the first piece using vacuum-assisted thermal bonding. The vacuum prevents formation of air-gaps in the bonding regions.

In addition to channels, other components, such as heaters, valves, and sensors (e.g., to detect specific conditions or components of the products of the device, such as pH, conductivity, or specific ions), may be fabricated in the device. Techniques are known in the art for the fabrication of such components. For heaters, resistive elements (e.g., metal or ceramic strips) may be molded into a device or evaporated or otherwise deposited onto the device. When a voltage is applied, the resistive element emits heat. Connections to external fluid sources or receptacles may be made by any standard means, e.g., Luer locks, compression fittings, and threaded fittings.

Chemical Derivitization

The surfaces of the device may be treated in order to ensure that the θ is greater (or lesser) than 90°. Alternatively, the device may be fabricated out of a material that provides the appropriate θ. Surface coatings whose θ may be changed, e.g., by the application of light or an electric field, may also be employed. Examples of such coatings include titanium oxide and polypyrrole. To reduce non-specific adsorption of cells or compounds released or formed during operation of the device onto the channel walls, one or more channel walls may also be chemically modified to be non-adherent or repulsive, such as a thin film coating (e.g., a monolayer) of commercial non-stick reagents, such as those used to form hydrogels. Additional examples of chemical species that may be used to modify the surfaces of a device include oligoethylene glycols, fluorinated polymers, organosilanes (e.g., $C_{12}H_8F_{17}SiCl_3$), thiols, poly-ethylene glycol, poly-vinyl alcohol, mucin, poly-HEMA, methacrylated PEG, and agarose. Charged

polymers may also be employed to repel or attract oppositely charged species. Surfaces of the device may also be treated in order to capture materials produced or released in the device, e.g., small molecules, membrane fragments, or proteins. Mixtures of surface coatings may also be employed, e.g., a hydrophobic coating and a coating capable of binding specific molecules, as long as the θ is maintained at an appropriate value, e.g., greater than 90°. The type of chemical species used for surface modification and the method of attachment will depend on the nature of the fluid in the channel, the nature of the walls, and the species being attached. Surface coatings may be covalently or non-covalently attached. Such surface modification techniques are well known in the art. The surfaces of the device may be functionalized before or after the device is assembled.

Operation

A device of the invention may be employed for any purpose requiring the contacting of two fluids, e.g., synthesis of species (e.g., in situ synthesis of sensitive, highly reactive, or hazardous reagents), assays (e.g., biochemical or cellular assays), labeling of species (e.g., proteins or cells), and destruction of species (e.g., lysis of cells or degradation of macromolecules). The devices may be employed in a variety of fields, such as medical diagnostics, environmental or quality control monitoring, and basic research. An exemplary use of the device of the invention is the lysis of one or more cells for biochemical analysis of the lysate. A device of the invention may be controlled using electric currents, and no supplementary devices outside the chip are necessary. Thus, the automation of the use of devices of the invention is highly feasable.

In general, the device is operated by filling each main channel, dividing the fluid columns in one or more of the main channels, and causing segments of fluid to come into contact via a channel connecting two or more main channels. Constrictions or sieves in the channels may be used to trap particles, e.g., cells, in the segments that are to be contacted. Such trapping may occur by flowing a liquid sample into the channel until a particle reaches the constriction, or once a particle is introduced into a channel, gravity could be used to move the particle to the constriction. Particle capture may also bc

achieved by other methods, such as dielectrophoretic trapping, capture using antibodies or other binding molecules fixed to surfaces or structures in the device, and polymer brushes. In one embodiment, the segments are forced into contact by reducing the pressure in the connecting channel relative to the main channels, e.g., by applying suction to the connecting channel. In another embodiment, the surface of the connecting channel can be altered to reduce the θ of the fluid to below 90°, and fluid will spontaneously flow into the connecting channel. Alternatively, an additive may be introduced into the fluid in order to lower θ, e.g., introducing a surfactant into an aqueous solution. The volumes contacted can range from 0.1 pL to 100 μL, typically in the picoliter range. Once contacted, fluids may then mix actively or passively. In one embodiment, active mixing is achieved by drawing fluid in the connecting channel into another channel and then forcing the fluid back into the third channel. This process may be repeated until a desired level of mixing is achieved. For example, the connecting channel may be in communication with a chamber. This chamber is normally heated and is far enough from the connecting channel or insulated such that a normal temperature is still maintained in the connecting channel. After the fluid division and contact, the heated chamber is allowed to cool. The pressure inside the chamber will reduce, and fluid will be pulled from the connecting channel towards the chamber. By reheating the chamber, fluid is pushed back into the connecting channel. The magnitude, frequency and rate of this back and forth movement of fluid can be controlled, e.g., by the current applied to a resistive heater in the chamber. Alternatively, the connecting channel is in communication with a syringe or any other device that can achieve a controlled change in pressure over the time scale of interest.

After contact, the contents of the mixture of the two fluids may be collected for further reaction, separation, storage, or analysis. Products formed or released by the contacting of fluids on the device may be bound by particles (e.g., magnetic or non-magnetic beads that contain specific or non-specific binding groups) in one or both of the fluids mixed. If a device having constrictions through which beads cannot pass is

employed, beads may be rinsed in the device by passing buffer through the channels. Alternatively, the products may be removed as a fluid or analyzed on the device.

In one embodiment, the fluids to be mixed have a θ with the channel surfaces of greater than 90°. The pressure needed to move such a fluid through a channel increases as the cross-sectional area of the channel decreases. The pressure necessary for pumping can be calculated using the surface tension of the fluid (σ), the contact angle (θ) as defined before, and the radius of curvature (r) inside the channel into which fluid is pumped ($P = 2 \times \sigma \cos(\theta)/r$). As a consequence, the movement of the fluid in a device can be controlled by controlling the pressure applied to the fluid and the cross-sectional area of the channels. Using an appropriate combination of θ, cross-sectional area, and pressure, fluids may be pressure pumped into a channel without substantially entering a connected channel. In addition, the introduction of constrictions in a channel can be used to prevent the passage of fluid beyond a defined point.

When fluids have been introduced into the main channels, a fluid divider may be employed to segment the fluid column in one or more main channels. The actuation of the fluid divider will depend on its nature. For example, for fluid dividers that require heat to cause fluid expansion into the main channel or bubble nucleation, a voltage is typically applied to a resistive heating element. A radiation source, such as a laser, may also be used as a heat source. Alternatively, a chemical reaction may be used to generate heat or evolve gas. For a fluid divider including a heater in an enclosed chamber, fluid in the main channels may remain divided even after cooling because of the hystersis in the advancing and receding θ. If the fluid divider introduces a fluid into the fluid column of the main channel, the fluids in the main channels are preferably saturated in the fluid used to divide them. The channel may be designed to direct the flow of the immiscible fluid in the channels. For example, constrictions in the main channels may be used to prevent or reduce immiscible fluid flow in a particular direction. For other fluid dividers, e.g., such a mechanical valves, the divider may be actuated mechanically, electrically, magnetically, or chemically in order to close the valves.

The devices of the invention may be employed in an array format, i.e., the presence of many individual devices in a larger structure. Employing a laser heating system, as described above, would allow for individual actuation of fluid dividers without the need for multiple electrical connections.

Fluids to be mixed in the device are preferably aqueous, but organic liquids or even gases may also be employed. When gases are employed, the device is filled with a liquid prior to introduction of the gases to prevent expansion of the gas through the device.

The following examples are intended to illustrates various features of the invention are not intended to be limiting in any way.

Example 1. Fabrication of a device of the invention.

A device of the invention was microfabricated using standard techniques. Channels were patterned in PDMS by casting the polymer on a mold of SU-8 photoresist photopatterned on glass. Three different layers of SU-8 were patterened on the same substrate in order to create structures with different heights that correspond to channels of different cross sectional dimensions in the PDMS slab. Micro-heaters were fabricated by patterning gold electrodes on glass. The PDMS slab and the glass with the electrodes were aligned and bonded using oxygen plasma. The surfaces of the channels (both the PDMS and the glass) were modified using fluorosilane and rendered hydrophobic. The assembled device contains a series of channels with different widths and heights as shown in FIG. 1, and in more detail in FIG. 2.

The dimensions of the device were as follows: channel **1**: 30 × 85 × 15 μm (w × l × h); constrictions **2** and **8**: 12.5 × 12.5 × 4 μm; channel **3**: 12.5 × 15 × 2 μm; chambers **4**, **5**, **9**, and **10**: 22 to 38 × 200 × 15 μm; channel **6**: 12.5 × 38 × 3 μm; channel **12**: 12.5 × 450 × 4 μm; chambers **7** and **11**: semicircles with radius 30 μm and 15 μm high; and the triangular region between channel **6** and channel **12** was 2 μm high.

Example 2. Lysis of a cell using the device of the invention.

Referring to FIGS. 1 and 2, an isotonic phosphate buffer solution (PBS) was introduced through inlet **A**. By applying moderate pressure (~ 20 kPa), the PBS was pushed through channel **1**, and the fluid was stopped at constriction **2**. The fluid also did not enter channel **3**, and, as a consequence, air was trapped in chamber **4**. A cell suspension of 3T3 mouse fibroblast or MOLT – T lymphoblasts was introduced through inlet **B**. The liquid column joined the PBS column while air was trapped in chamber **5**. The liquid would not pass through channel **6** unless too high a pressure was applied (~ 80 kPa). While fluid could flow through constriction **2**, cells larger than the constriction were trapped in chamber **7**. Similarly, the lysing solution (3 M guanidine thiocyanate in water) was introduced through inlet **C** and pushed through constriction **8**, trapping air in chambers **9** and **10**. When the desired number of cells (one or more) was trapped in chamber **7**, a pulse of current of controlled duration and intensity was applied to the heaters, and the air trapped in channels **3**, **5**, **9** and **10** expanded into the nearby channels, separating the fluid columns into three segments each. A cell was trapped in the middle segment, corresponding to chamber **7**, still separated from the lysing solution separated in chamber **11** (FIG. 2). When suction is applied through channel **12**, fluid from both chambers **7** and **11** is pulled into channel **6**, and the two liquids contacted and were mixed by diffusion. This contact lead to the release of the cell contents in the isolated space of chambers **7** and **11** and channel **6**. Beads may be introduced simultaneously with the cells, and specific components of the cell can be captured on the surface of the beads and kept in place during subsequent washing steps. The content of the lysing chambers could also be removed through channel **12** and taken out of the device or moved into a different section of the device for further analysis.

Other Embodiments

All publications, patents, and patent applications mentioned in the above specification are hereby incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in

the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art are intended to be within the scope of the invention.

Other embodiments are in the claims.

What is claimed is:

CLAIMS

1. A device for contacting two volumes of fluid, the device comprising:

(a) a first channel having a fluid inlet and a first fluid divider for dividing the fluid in the first channel into discrete segments;

(b) a second channel having a fluid inlet and a second fluid divider for dividing the fluid in the second channel into discrete segments; and

(c) a third channel connecting the first and second channels.

2. The device of claim 1, wherein the first or second fluid divider comprises one or more sources for an immiscible fluid.

3. The device of claim 2, wherein the source for the immiscible fluid is an enclosed chamber connected to the first or second channel.

4. The device of claim 1, further comprising a constriction in the first channel.

5. The device of claim 1, further comprising a constriction in the second channel.

6. The device of claim 1, wherein the contact angle of an aqueous solution with the surface of the first channel is greater than 90°.

7. The device of claim 1, wherein the contact angle of an aqueous solution with the surface of the second channel is greater than 90°.

8. The device of claim 1, wherein the contact angle of an aqueous solution with the surface of the third channel is greater than 90°.

9. The device of claim 1, wherein the contact angle of an aqueous solution with the third channel can be reduced to less than 90° by the application of light or an electric field.

10. The device of claim 1, wherein the first or second channel comprises a chamber having a volume of 0.1 pL – 100 μL.

11. A method of contacting two fluids, the method comprising the steps of:

(a) providing a device comprising:

(i) a first channel having a fluid inlet and a first fluid divider for dividing the fluid in the first channel into discrete segments;

(ii) a second channel having a fluid inlet and a second fluid divider for dividing the fluid in the second channel into discrete segments; and

(iii) a third channel connecting the first and second channels;

(b) pumping a first fluid through the inlet into the first channel and pumping a second fluid through the inlet into the second channel;

(c) employing the first fluid divider to divide the first fluid into a plurality of segments to form a first segment connected to the third channel;

(d) employing the second fluid divider to divide the second fluid into a plurality of segments to form a second segment connected to the third channel; and

(e) contacting the first and second segments via the third channel.

12. The method of claim 11, wherein the first fluid divider comprises one or more sources for fluid immiscible in the first fluid, and wherein step (c) comprises introducing the immiscible fluid into the first channel to divide the first fluid into a plurality of discrete segments.

13. The method of claim 12, wherein the source for the immiscible fluid is an enclosed chamber connected to the first channel.

14. The method of claim 11, wherein the second fluid divider comprises one or more sources for fluid immiscible in the second fluid, and wherein step (d) comprises introducing the immiscible fluid into the second channel to divide the second fluid into discrete segments.

15. The method of claim 14, wherein the source for the immiscible fluid is an enclosed chamber connected to the second channel.

16. The method of claim 11, wherein the first fluid comprises a cell, and the second fluid comprises a lysis solution capable of lysing the cell.

17. The method of claim 16, wherein the first segment in step (c) comprises the cell, and the second segment in step (d) comprises the lysis solution, wherein the cell is lysed in step (e).

18. The method of claim 16, wherein the device further comprises a constriction in the first channel, and wherein in step (b), the cell does not flow through the constriction.

19. The method of claim 11, wherein the first fluid has a contact angle of greater than 90° with the surfaces of the first and third channels.

20. The method of claim 11, wherein the second fluid has a contact angle of greater than 90° with the surfaces of the second and third channels.

21. The method of claim 11, wherein the volume of the first or second segment is 0.1 pL – 100 μL.

22. The method of claim 11, wherein said contacting in step (e) occurs by reducing the pressure in the third channel relative to the first and second channels.

23. The method of claim 11, wherein said contacting in step (e) occurs by reducing the contact angle of the first and second fluids with the third channel to less than 90°.

24. The method of claim 11, wherein step (e) occurs prior to step (b) or step (c).

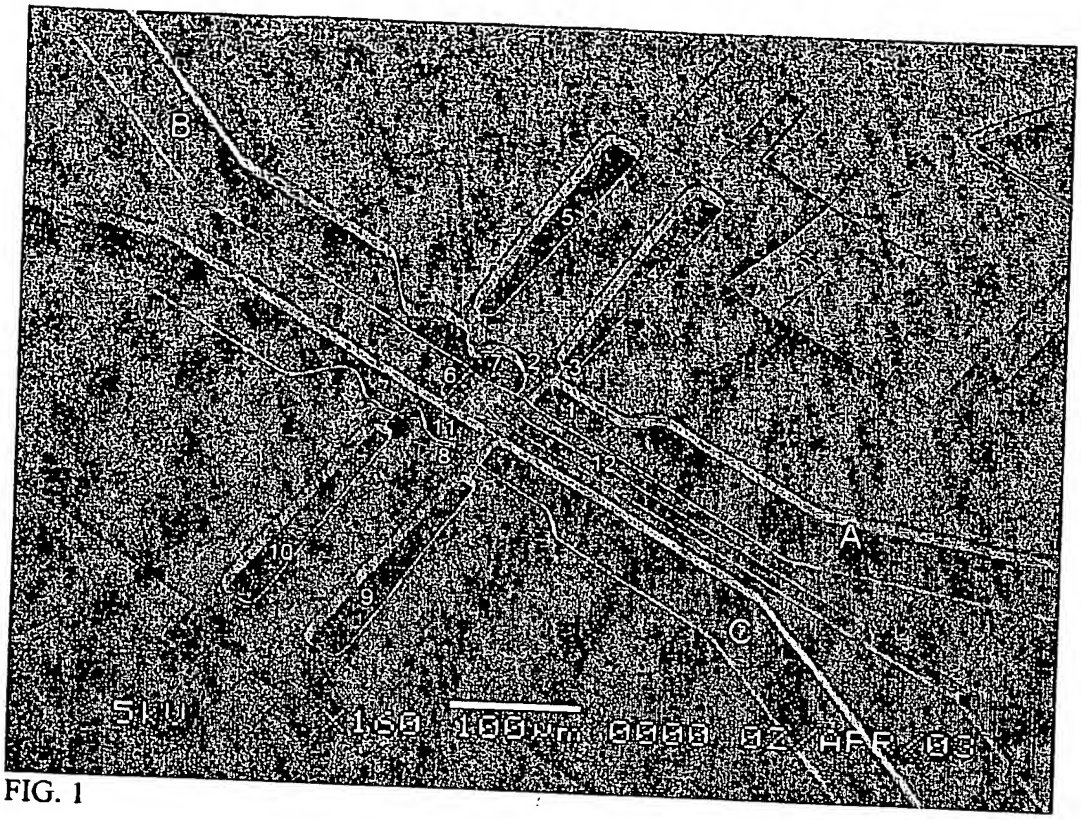

FIG. 1

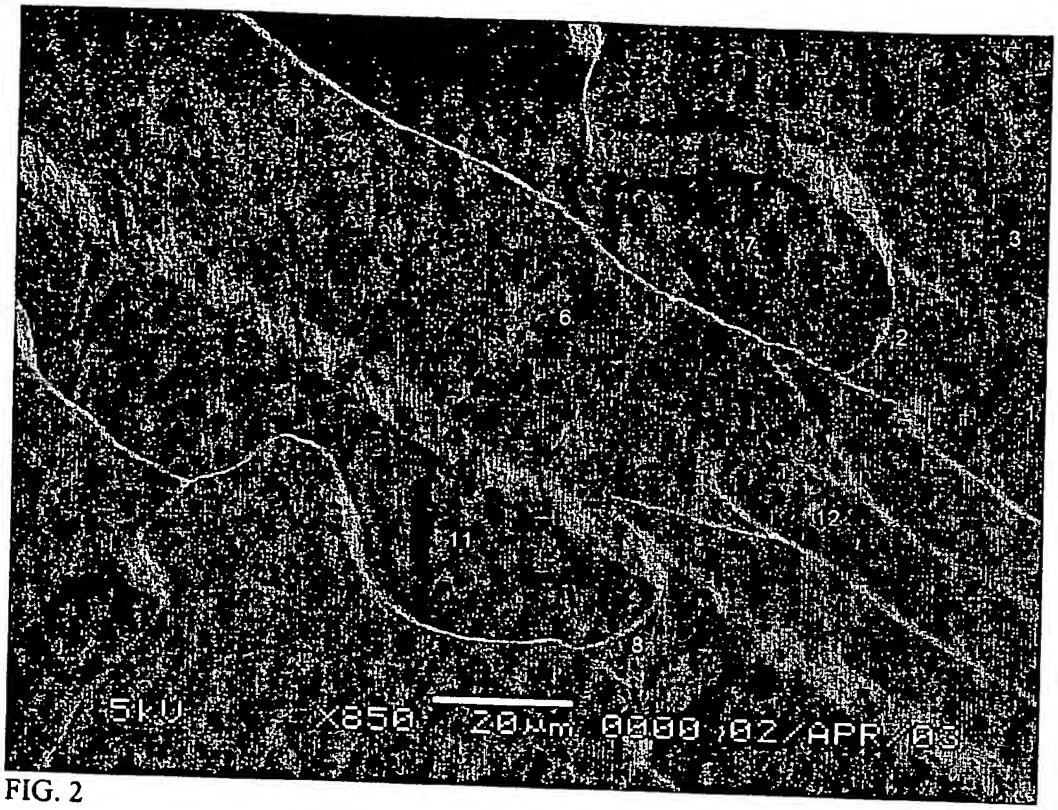

FIG. 2

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